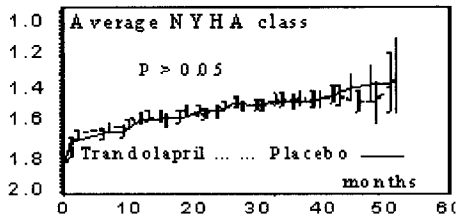
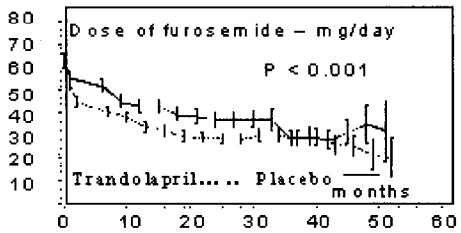
NYHA classes in patients with left ventricular dysfunction after MI. Trandolapril resulted in significantly lower furosemide consumption.





1184-81

Elevated Plasma Xanthine Oxidase Activity in Chronic Heart Failure: Source of Increased Oxygen Radical Load and Effect of Allopurinol in a Placebo Controlled, Double Blinded Treatment Study

Wolfram Doehner, Margaret M. Tarpey, Darrell V. Pavitt, Aidan P. Bolger, Roland Wensel, Stephan von Haehling, David A. Reaveley, Stefan D. Anker, National Heart & Lung Institute, London, United Kingdom, University of Alabama, Birmingham, AL

Background: Elevated xanthine oxidase (XO) activity contributes to production of reactive oxygen species. Hyperuricemia is common in chronic heart failure (CHF), however, enzyme activity of circulating plasma XO has not been studied in CHF. We hypothesised, that plasma XO activity is elevated in CHF compared to healthy controls, paralleled by increased free radical load, and that XO inhibition with allopurinol decreased both. As a marker of free radical load serum allantoin was measured, which is generated in the humans exclusively via non-enzymatic oxygen radical dependent urate oxidation.
Methods: In 67 CHF patients (mean age 65±10y, NYHA 2.4±0.7, peak VO_2 18.6±6.8 mL/kg/min) and 15 controls (age 33±10y) we measured plasma XO activity (HPLC), uric acid (UA), and allantoin (gas chromatography-mass spectrometry). In 17 CHF patients with known hyperuricemia (UA 517±95µmol/L), the effect of allopurinol (300mg od for 1 week) was tested in a placebo controlled double-blinded, cross-over study.
Results: CHF patients were hyperuricemic (UA 481±143µmol/L; normal range 210-440 µmol/L) and had increased plasma XO activity (7.01±0.60 vs 0.89±1.23 vs µU/ml; p<0.001). The upper limit of normal was defined as 3.35 µU/ml XO (normal mean value +2SD). All but one control and 11 patients (16%) had normal plasma XO activity, but 56 patients (84%) had elevated XO activity (Chi^2 p<0.0001). Allantoin was elevated in CHF compared to reference control values (41.0±26.2 vs 13.4±1.6 µmol/L). In the double-blinded allopurinol treatment study, plasma XO activity was reduced in all 17 patients by 49% (from 4.35± 2.7± to 2.25±1.3, p<0.001). Allopurinol also reduced allantoin levels by 18% (from 25.7±4.1 to 21.1±1.0, p<0.02).
Conclusion: The activity of circulating plasma XO is elevated in patients with CHF. Treatment with allopurinol results in reduction of XO activity that is paralleled by lower allantoin indicating reduced oxygen radical load. Thus, a potentially new therapeutic option emerges to reduce oxygen radical load in CHF.

1184-82

Chronic Monotherapy With Extended Release Metoprolol Succinate Attenuates mRNA Gene Expression for MMP2 and MMP9 in Dogs With Heart Failure

Sharad Rastogi, Ramesh C. Gupta, Sudhish Mishra, Hideaki Morita, George Suzuki, Sidney Goldstein, Hani N. Sabbah, Henry Ford Health System, Detroit, MI

Background: Accumulation of collagen in the cardiac interstitium or "reactive interstitial fibrosis" (RIF) occurs in heart failure (HF) and contributes to LV dysfunction and remodeling. Matrix metalloproteinases (MMPs) are upregulated in HF and contribute to RIF. We previously showed that therapy with extended release metoprolol succinate (ER-MET) significantly reduces RIF in dogs with HF. In this study, we examined the effects of chronic therapy with ER-MET on gene expression of MMP2 and MMP9 (gelatinases) and on tissue inhibitors of MMPs (TIMPs) in LV of dogs with microembolization-induced HF.
Methods: Total RNA was isolated from LV tissue of 14 dogs with HF randomized to 3 months therapy with ER-MET (50 mg, once daily, n=7) or to no therapy at all (n=7) and from LV of 6 normal (NL) dogs. mRNA expression for MMP2 and 9 and TIMP1 and 2 was measured using reverse transcriptase polymerase chain reaction and bands quantified in densitometric units. **Results:** Results are shown in the table. There were no differences in expression of TIMP1 and 2 among the 3 study groups. Expression of MMP2 and 9 was increased in untreated-HF dogs compared to NL. ER-MET significantly reduced this increase in expression of MMP2 and 9. **Conclusions:** In dogs with HF, mRNA gene expression of TIMPs is unchanged while expression of MMP2 and 9 is increased. Therapy with ER-MET does not influence TIMPs but reduces expression of MMP2 and 9, a finding consistent with reduced RIF following chronic therapy with the ER-MET.

	NL	HF-Untreated	HF + ER-MET
TIMP1	2.33 ± 0.35	2.93 ± 0.46	1.90 ± 0.38
TIMP2	1.31 ± 0.04	1.39 ± 0.02	1.35 ± 0.06
MMP2	1.30 ± 0.18	2.04 ± 0.11*	1.39 ± 0..08^
MMP9	1.22 ± 0.14	2.50 ± 0.04*	1.15 ± 0.10^

*=P<0.05 vs. NL; ^=P<0.05 vs. HF-Untreated

1184-83

Dietary Fish Oil Supplementation Improves Endothelial Function in Patients With Congestive Heart Failure

David R. Morgan, Lana J. Dixon, Colm G. Hanratty, Sinead M. Hughes, William J. Leahey, Naglaa A. El-Sherbeeny, G. Dennis Johnston, Gary E. McVeigh, Queen's University Belfast, Belfast, United Kingdom

Background
Systemic vasoconstriction and reduced peripheral perfusion are hallmarks of congestive heart failure (CHF). Endothelial dysfunction is a frequent finding in CHF and is associated with reduced bioavailability of the vasodilator autocoid nitric oxide (NO). Omega-3-fatty acids (fish oils) have been shown to have beneficial effects on endothelial function and vascular responses in a number of vascular diseases which may be secondary to increased NO bioavailability. We conducted a study to establish whether addition of omega-3- fatty acids to background therapy in patients with CHF would improve endothelial dysfunction.
Methods
20 patients with grade II and III CHF (15 male) mean age 73 were recruited. Sodium nitroprusside (SNP) (6, 9, 12nmol/min) and acetylcholine (ACH) (120,180, 240nmol/min) were infused into the non-dominant brachial artery. Forearm blood flow (FABF) responses assessed by venous occlusion plethysmography. Patients received fish oil or olive oil drink for 6 weeks in a double blinded randomised cross over trial with assessment of FABF at baseline and after each treatment.
Results
See table
Conclusion
Dietary fish oil significantly improved endothelial function as assessed by FABF responses to ACH. There are several possible mechanisms by which fish oils could potentially improve endothelial function in CCF. Further studies are required to attempt to elucidate the mechanism for this improvement and to establish whether will be associated with improved outcomes.

Forearm Blood Flow responses to infusion of SNP and ACH (values are arbitary units) (*=p<0.01)

	SNP	ACH
Baseline 1	14.22 (10.93,17.51)	7.95 (4.81,11.80)
Fish oil	11.66 (8.17,15.15	11.27* (7.31,15.23)
Baseline 2	13.55 (10.74,16.36)	7.68 (4.95,10.41)
Olive oil	14.38 (11.67,17.09)	7.27 (4.66,9.88)

POSTER SESSION

1185 Cardiac Transplantation: Cellular Mechanisms and Rejection

Tuesday, April 01, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: 1:00 p.m.-2:00 p.m.

1185-59

Acute Rejection in Human Heart Transplantation: Identification and Characterization of Two Important Markers (MIP-1 β and VE-Cadherin)

Ana L. S. Roussoulieres, Olivier Raisky, Lara Chalabreysse, George Dureau, Catherine Cerutti, Pascale Boissonnat, Laurent Sebbag, Jean-Paul Gare, Jean-François Obadia, Jean Ninet, Olivier Bastien, Françoise Thivolet-Bejui, John L. McGregor, Hôpital Cardiologique Louis Pradel, Lyon, France, INSERM, Lyon, France

Background: An extensive number of molecules are involved in acute rejection (AR) following heart transplantation. We have previously identified by DNA arrays, in a murine model of heterotopic heart transplantation, a number of genes implicated in allograft AR. The expression of 2 of these genes, MIP-1β and VE-Cadherin, was investigated in the present study as potential new markers of AR in cardiac tissue following human heart transplantation.
Methods: We have previously studied the expression profile of genes involved in AR

after heterotopic heart transplantation in a murine model. Using the technique of macroarrays and immunohistochemistry, we have shown that MIP-1β was over expressed and that VE-Cadherin was under expressed in the endothelial cells (ECs) of rejecting allografts. In this study, routine endomyocardial biopsies were performed following human heart transplantation. Cardiac tissues were embedded in paraffin for routine histologic analysis. Specimen was graded for AR using the "ISHLT" criteria. Immunohistochemical staining for the expression of MIP-1β and VE-Cadherin was performed in cardiac tissues showing either no rejection (n=10), rejection grade IB (n=10), or rejection grade IIIA (n=10).
Results: MIP-1β was strongly expressed (+++) on ECs in heart tissues showing an AR grade IIIA when compared to heart tissue showing grade IB (++) AR or cardiac tissues with no AR (+). VE-Cadherin was detected as a thin, linear staining on ECs in cardiac tissues showing no rejection (+++ or strongly positive). In contrast, the VE-Cadherin staining was weak (+ or weakly positive) or completely absent on ECs present in biopsies showing AR (grade IB and IIIA).
Conclusions: We have identified and validated for the first time 2 genes (MIP-1β and VE-Cadherin) present in ECs lining the vessel walls, as markers of AR in human cardiac tissues. MIP-1β, a chemokine, induces chemotaxis and adhesion of T-cells on ECs; VE-Cadherin, an endothelial-specific membrane protein responsible for the endothelial cell-cell adhesion, plays a key role in the migration of lymphocytes into myocardial tissues. Validated genes derived from the murine model can be used as potential targets in AR in human heart transplantation.

1185-60 Post-Transplant Cardiac Rejection Monitoring With and Without Routine Biopsy Screenings: Comparison of Two Different Surveillance Strategies

Michael Dandel, Johannes Müller, Manfred Hummel, Rudolf Meyer, Susanne Kapell, Roland Hetzer, Deutsches Herzzentrum, Berlin, Germany

Background: Rejection surveillance is extensively based on routine endomyocardial biopsy (EMB) screenings. Nevertheless, routine EMBs are distressing to the patients and risky. To verify the possibility to replace routine EMB screenings by efficiently timed diagnostic EMBs, we compared the diagnostic efficiency of routine EMBs with that of a combined, mainly non-invasive rejection surveillance-strategy, in which EMBs were performed optionally, only in patients suspected for rejection.
Methods: Two groups of patients underwent different rejection surveillance strategies during their first post-transplant year. In group A (n =76) we performed a telemetric monitoring of the intramyocardial electrogram (IMEG) from a dual-chamber pacemaker. Overnight IMEG changes were analyzed on daily printouts. Additional pulsed-wave tissue Doppler (PW-TD) wall motion analyses were performed daily during hospitalization and after discharge, at each ambulatory examination. In group B (n =22), additionally to IMEG recordings and PW-TD examinations, independent routine EMB screenings were performed at predefined time intervals.
Results: In group A the mean number of EMBs per patient (1.45 ±1.25) was 88.9% lower than the number of routine EMBs performed in each patient of group B. In group A, 21.1% of the patients had no relevant IMEG and/or PW-TD changes and therefore no EMBs. The average numbers of rejection therapies per patient performed in group A (0.74 ±0.71) and group B (0.77 ± 0.75), as well as the number of morphological significant (ISHLT grade > 2) rejection episodes per patient (0.16 ± 0.15) in group A and 0.14 ± 0.13 in group B) were similar. In group B 93.6% of the routine EMBs had no therapeutic consequences. In group A, 50.9% of rejection episodes, suspected by IMEG and/or PW-TDI changes and confirmed by EMBs, were clinically relevant and needed antirejection treatment. No patient died during the study.
Conclusions: Non-invasive rejection surveillance based on IMEG recordings and tissue Doppler wall motion analyses in combination with diagnostic EMBs allow a reliable, efficient and save monitoring even during the first post-transplant year, without unnecessary and distressing routine EMBs.

1185-61 The Utility of Surveillance Endomyocardial Biopsies in Detecting Cellular Rejection in Pediatric Heart Transplant Patients

Daniel S. Levi, Adam S. DeConde, Caron Burch, Juan C. Alejos, Glenn T. Wetzel, University of California, Los Angeles, Los Angeles, CA

Background: Routine surveillance endomyocardial biopsies (EMB) are commonly used to screen for cellular rejection in pediatric heart transplant patients. With advances in immunosuppression, the benefit of EMB in asymptomatic pediatric heart transplant patients is unclear.
Methods: After orthotopic heart transplant (OHT), surveillance EMBs were routinely performed on all pediatric OHT patients with decreasing frequency. All biopsy specimens were reviewed by a cardiac pathologist, and graded according to International Society for Heart and Lung Transplantation (ISHLT) guidelines. A retrospective review of consecutive EMBs performed at our institution from January 1995 to September 2002 was conducted. The echocardiogram results, clinical history and treatment changes at the time of every biopsy were also recorded.
Results: Results of 866 EMBs from 91 patients were reviewed. Two hundred and thirty-seven EMBs (23.9%) were performed within thirty-days of OHT, 394 EMBs (45.5%) were performed between one month and one year from OHT, and 265 EMBs (30.6%) were performed more than 1 year after OHT. Of all EMBs, 1.39% were ISHLT grade 2 or higher, 3.58% were grade 1B, 19.4 % were grade 1A, and 74.6% were grade 0. Six of the EMB were unable to be interpreted because of insufficient tissue. Of the twelve patients in whom the EMB was read as grade 2 or higher, six were less than one month from OHT and asymptomatic. The other six patients with greater than 1B cellular rejection presented for biopsy because of symptoms and had abnormal function on echocardiogram. Of the 820 EMB performed in asymptomatic patients more than one month from OHT, there were no episodes of cellular rejection greater than 1B. There were 21 asymptomatic patient biopsies (2.56%) with grade 1B rejection. All grade 1B rejection detected by surveillance EMB resolved in both treated and untreated cases.
Conclusion: EMB should only be used to screen for cellular rejection in the first month after pediatric heart transplantation. For pediatric patients more than thirty days after OHT, EMB has failed to reveal significant episodes of cellular rejection in asymptomatic patients. The utility of surveillance EMB to detect humoral rejection was not assessed.

1185-84 Rejection Surveillance Late After Heart Transplantation

Michael Dandel, Manfred Hummel, Susanne Kapell, Rudolf Meyer, Hans B. Lehmkuhl, Roland Hetzer, Deutsches Herzzentrum Berlin, Berlin, Germany

Although routine endomyocardial biopsies (EMBs) continue to detect cardiac rejection (CR) beyond the first post-transplant year, their need for late CR surveillance is controversial. However, late CRs are associated with both graft failure and allograft coronary disease (ACD). To provide appropriate CR surveillance during late post-transplant periods, we assessed the usefulness of non-invasive screenings for both CR diagnosis and effective use of EMBs.
Methods: In 130 patients (post-transplant times: 2– 15 years) monitored routinely by tissue Doppler imaging (TDI), we compared the diagnostic efficacy of routine EMBs (performed unrelated to TDI results) with that of diagnostic EMBs (timed by TDI). Routine EMBs were performed in 98 patients during annual follow-up catheterizations. Diagnostic EMBs, conducted whenever TDI detected left ventricular wall motion alterations (prolongation of relaxation time and/or reduction of systolic and/or diastolic peak velocities), were performed in 32 patients.
Results: Most routine EMBs (89.9%) were ISHLT grade 0 and TDI performed before showed no CR relevant changes. CRs grade 1A and 1B were shown in 8.1% of routine EMBs. Two routine EMBs (2%), obtained from 2 asymptomatic patients with TDI changes, showed relevant CRs grade 3A. Among the 38 diagnostic EMBs performed due to TDI alterations in 32 patients, 7 (18.4%) were ISHLT grade 0, but in 5 cases the coronary angiogram showed either new appearance or aggravation of ACD. The other 31diagnostic EMBs showed cellular CRs of different degrees (32.3% 1A and 1B, 9.8% grade 2, 57.9% 3A and 3B). Vascular reactions were detectable in 22 diagnostic EMBs. Reduction with >15% of systolic velocity Sm, evident in 81.8% of all patients with CR, was shown in all patients with clinically relevant CRs (ISHLT ≥ grade 2 plus 1A and 1B accompanied by hemodynamic deterioration and/or vascular rejections).
Conclusions: Routine annual EMBs detect only a fraction of relevant CRs which occur late after transplantation. Serial TDI screenings followed by diagnostic EMBs, whenever relevant wall motion alterations are detected, increase the efficacy of CR diagnosis and provide a tempting strategy for late post-transplant CR surveillance.

1185-85 Noncultured Autologous Skeletal Muscle Cells Can Successfully Engraft in Ovine Myocardium

Nicolas Borenstein, Patrick Bruneval, Mehrak Hekmati, Christophe Bovin, Luc Behr, Christian Pinset, François Laborde, Didier Montarras, CERA - Centre d' Experimentation et de Recherche Appliquee, Paris, France

Background: The concept of myogenic cell transplantation into the myocardium, known as cellular cardiomyoplasty (CCM), is based on the contribution of exogenous cells to replace lost or altered cardiomyocytes in order to restore functional performances of the heart. There is a large body of evidence showing that CCM performed with skeletal muscle cells can improve cardiac function in ischemic heart disease as well as dilated cardiomyopathy on numerous animal models. Most research teams have addressed autologous CCM in a three phase process : biopsy, ex vivo cell culture/expansion and surgical or catheter based cell delivery. Considering the potential benefit of using non cultured muscle cells (little time, lower cost, reduced risk of contamination), we investigated the feasibility of grafting cells obtained directly after enzymatic dissociation of skeletal muscle biopsies in ovine myocardium.We hypothesized that those non cultured muscle cells would massively engraft. Methods: Autologous intramyocardial skeletal muscle cells implantation was carried out in 8 sheep. A skeletal muscle biopsy (about 10 g) was explanted from each animal. The sheep were left to recover over approximately three hours and reanesthetized when the cells were ready for the implantation. A left fifth intercostal thoracotomy was performed and 10 epicardial injections of the muscle preparation (between 10 and 20 million cells) were carried out. All sheep were euthanized 3 weeks after myocardial implantation. Immunohistochemistry was performed with monoclonal antibodies to a fast skeletal isoform of myosin heavy chain. Results: Skeletal myosin heavy chain expression was detected in all slides at 3 weeks after implantation in 8 of 8 animals, confirming engraftment of skeletal muscle cells. Massive areas of engraftment (from 2 to 9 mm in diameter) or discrete loci were noted within the myocardial wall. Conclusions: In conclusion, our results indicate that non-cultured skeletal muscle cells can successfully and massively engraft in ovine myocardium. Thus, skipping the cell culture expansion phase is feasible and could become a promising option for cellular cardiomyoplasty.

1185-86 Combination of Mesenchymal Stem Cell Transplantation and Angiogenic Gene Transfer for Myocardial Regeneration and Therapeutic Angiogenesis

Tae-Jin Youn, Hainan Piao, Young-Hwa Kim, So-Young Choi, Jin-Sook Kwon, Bo-Ra Son, Dong-Woon Kim, Seung-Taik Kim, Myeong-Chan Cho, College of Medicine, Chungbuk National University, Cheongju, South Korea

Bone marrow-derived stem cells including mesenchymal stem cells (MSCs) have attracted attention as potential platforms for the delivery of therapeutic genes. Lentiviral vectors are promising tools for the development of gene therapy since they can transduce both quiescent and dividing cells. We have previously demonstrated that MSCs can be differentiated into cardiomyocytes and restoration of blood flow is crucial for the fate of